

CLAIMS

1. A method for generating calibration data for absolute quantitation of RNA by RT-PCR, the method comprising: (a) providing a synthetic oligonucleotide comprising an amplicon and a promoter sequence located 3' relative to the amplicon; (b) synthesizing complementary RNA (cRNA) by in vitro transcription of the oligonucleotide; (c) quantitatively assaying the cRNA by an independent method; and (d) generating calibration data using a known quantity of the cRNA.

10

5

- 2. The method of claim 1, wherein the promoter sequence is a bacteriophage promoter sequence.
- 3. The method of claim 2, wherein the bacteriophage promoter sequence is a T7 promoter sequence.
 - 4. The method of claim 3, wherein the T7 promoter sequence consists essentially of 5'CCTATAGTGAGTCGTATTA 3' (SEQ ID NO:1).
- 5. The method of claim 1, further comprising a 5' flanking sequence consisting of 2 to 20 nucleotides adjacent to the amplicon.
 - 6. The method of claim 5, wherein the 5' flanking sequence consists of 8 to 12 nucleotides.

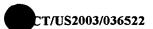
25

- 7. The method of claim 5, wherein the 5' flanking sequence comprises a poly T tail.
- 8. The method of claim 1, wherein the synthetic oligonucleotide further comprises a 3' flanking sequence consisting of 2 to 20 nucleotides between the amplicon and the promoter sequence.

5

15

25



- 9. The method of claim 8, wherein the 3' flanking sequence consists of 8 to 12 nucleotides.
- 10. The method of claim 1, wherein the length of the amplicon is 30 to 70 nucleotides.
- 11. The method of claim 10, wherein the length of the amplicon is 40 to 60 nucleotides.
- 12. The method of claim 1, wherein the length of the synthetic oligonucleotide is 60 to 140 nucleotides.
 - 13. The method of claim 12, wherein the length of the synthetic oligonucleotide is 70 to 130 nucleotides.
 - 14. The method of claim 13, wherein the length of the synthetic oligonucleotide is 80 to 120 nucleotides.
- 15. The method of claim 14, wherein the length of the synthetic oligonucleotide is 90 to 110 nucleotides.
 - 16. A method for determining the abundance of nucleic acid molecules comprising an amplicon in a test sample, the method comprising:
 - (a) providing a synthetic oligonucleotide comprising an amplicon and a promoter sequence located 3' relative to the amplicon;
 - (b) synthesizing cRNA by in vitro transcription of the oligonucleotide;
 - (c) producing a dilution series using the cRNA;
 - (d) synthesizing single stranded cDNA by reverse transcription of the cRNA;
 - (e) generating RT-PCR calibration data;
- 30 (g) obtaining RT-PCR test sample data from the test sample; and
 - (h) comparing the PCR test sample data to the PCR calibration data.

5



- 17. The method of claim 16, further comprising quantitating the cRNA.
- 18. The method of claim 17, further comprising mixing the cRNA with heterologous RNA before synthesizing the single stranded cDNA.